

## Analysis of Covalently Cationized Polystyrenes Using Liquid Chromatography and Mass Spectrometry\*

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### Introduction

Production of covalently charged polymers of known molecular mass (MM) is important in determining the ability of mass spectrometry (MS) to provide exact MM and relative molecular mass distribution (MMD) information of synthetic polymers. The detected ions are preformed; therefore, no ionization bias due to dependence on salt concentration and ionizable functional groups occurs within the mass spectrometer. Comparison of the mass spectra of polystyrene samples, ionized by both traditional metal cationization and covalent cationization reveals that covalent cationization increases signal-to-noise and provides more reproducible data. However, quantitation is far from absolute. Covalently charged products are contaminated with excess reagents and side products and can be difficult to purify by precipitation. Purification is needed before further analysis can be carried out. Liquid chromatography (LC) is a widely used technique for organic separation and purification. Matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS) after LC is important in determining the presence of polymer in fractions collected from a column and provides insight into the separation efficiency. Analysis by nuclear magnetic resonance (NMR) after purification can support data that is readily obtained by MS and rapidly screen for sample purity.

### Method

Atom transfer radical polymerization (ATRP) was used to prepare bromine terminated polystyrene (Br-PS). The product was recovered and reacted with tributyl phosphine (TBP) to yield the covalently cationized polymer (Figure 1). The column was prepared by the slurry method using silica mixed in mobile phase. One hundred milligrams of sample, dissolved in the mobile phase, was loaded onto the column. The sample was eluted with 500 mL of mobile phase, and fractions were collected in pre-weighed 20 mL vials. The solvent was allowed to evaporate, and then the vials were re-weighed. Fractions that showed a significant weight change were analyzed by MALDI-TOF-MS with a mass accuracy of  $\pm 0.2$  Da for end group determination and NMR for the qualitative detection of TBP.

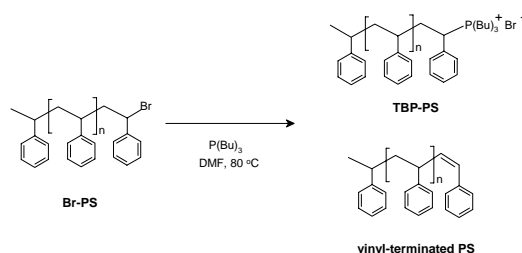


Figure 1: Reaction of Br-PS with TBP yields TBP-PS and vinyl-terminated PS

### Results and Discussion:

A normal phase preparative LC-column was used to separate a model system, which was composed of a 2100 g/mol standard polystyrene (PS 2100) spiked with TBP. The molecular mass PS standard corresponds with the molecular mass of the actual sample being studied. Fractions were collected to determine separation efficiency of the column based on mobile phase polarity. When toluene was used as the mobile phase, MALDI-MS detected polystyrene in several fractions. NMR analysis of the same fractions showed no free TBP present. However, when THF was used as the mobile phase, TBP was present in each fraction that contained polymer signifying co-elution of the polymer and TBP. Based on these results, it was decided to employ the column using toluene as the mobile phase on the actual sample.

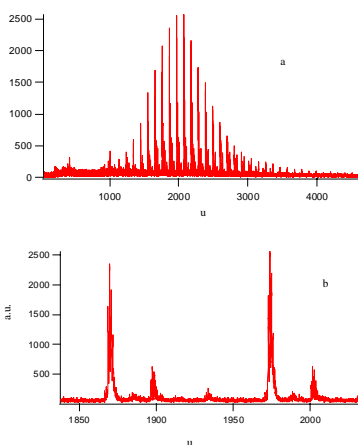
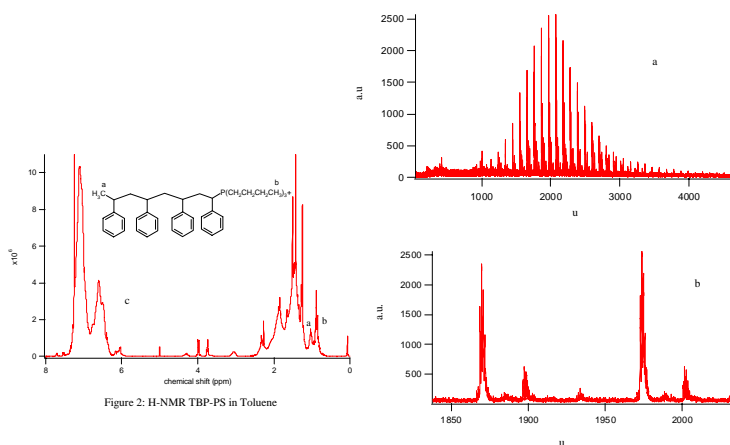
LC separation of the actual sample was prepared in the same fashion as the column used for the PS 2100 standard. Several fractions showed a significant mass change, which mimics the behaviour of the model system. The

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fraction that showed the largest mass change was analyzed by MALDI-MS and NMR. No TBP or polystyrene tributyl phosphine terminated (TBP-PS) was observed by NMR for the fraction. The MALDI- MS analysis also confirmed the absence of covalently cationized polystyrene. The only distribution detected above noise was that of a sodium cationized vinyl terminated product. The total mass collected was 23 mg, which represents a 43 % recovery. All data indicating that the column was not successful.

Three other columns were packed and run with different solvent mixtures in an effort to change the polarity enough to separate the polymer from the contaminants. The solution mixtures included 95:5, 90:10, and 80: 20 by volume mixtures of toluene to THF. No polymer of interest was observed during the MS analysis of the collected fractions of the aforementioned columns. The functionalized polymer was possibly retained or absorbed to the column packing or eluted so late in the separation that the fractions were never collected, i.e., very long retention time. Therefore, the columns were not acceptable for analytical separation.

Finally, due to the failure of solvent mixtures in separating the components of the sample mixture, it was decided to vary the solvent composition in a single LC experiment because of the success of toluene in removing the non-polar portion of the synthesized product. Varying the solvent composition during a signal chromatographic separation would hopefully remove products in order of increasing polarity allowing for complete recovery and characterization of all products present in the sample. The sample was then eluted with 200 mL of 100 % toluene and collected in a beaker. The solvent was then switched to a 50:50 mixture by volume of toluene to THF and 200 mL was passed through the column and collected in a different beaker. The mobile phase was then changed to 100 % THF and 200 mL was used for the final stage of elution and collected in a third beaker. NMR and MS were performed on the residues obtained from each solvent elution. The qualitative NMR of the 100 % toluene fraction (Figure 2) revealed a peak at 0.85 ppm (peak b), which is indicative of the TBP end group. The theoretical ratio of peak b to peak a (terminal methyl on initiating end) should be 3:1. The calculated ratio is from the NMR is approximately 4:1, which suggest that the purification was a success. The reason for the discrepancy between the calculated and the observed ratios is that only 32 scans were performed yielding poor signal-to-noise ratio. The NMR also shows the presence of vinyl terminated polymer (peak c). The ratio of peak a to peak c in Figure 2 is 1:1, suggesting that a large portion of the sample is vinyl terminated. Mass spectral results (Figure 3a) show a main distribution (Figure 3b) that is TBP-PS with the expected structure as calculated for the original analysis from the sample before purification. The second, small distribution is the product with the vinyl termination. This distribution is of significant less intensity due to ionization efficiency of the two structures. The vinyl-terminated polymer has to scavenge free, naturally occurring sodium while the covalent cationized polymer has 100 % efficiency. The mass of the non-polar residue collected was 21 mg giving a 38 % recovery of the total sample, suggesting that the sample is less then 38 % pure.



## Conclusion

Changing solvent polarity during elution seems to allow for the collection of all products with varying end groups, no matter how minor. This information lays the groundwork for large scale separation by end groups and molecular weight which could be applicable to the formulation of blends by having pure starting materials and to synthesis by purification before the next step.